CALIFORNIA MOSQUITO-BORNE VIRUS SURVEILLANCE & RESPONSE PLAN

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Introduction

California has a comprehensive mosquito-borne disease surveillance program that has monitored mosquito abundance and mosquito-borne virus activity since 1969 (Reeves et al. 1990). Surveillance and interagency response guidelines have been published previously by the California Department of Health Services (Walsh 1987) and the Mosquito and Vector Control Association of California (Reisen 1995). The detection of West Nile (WN) virus in New York, a virus not recognized in the Western Hemisphere prior to 1999, prompted the review and enhancement of existing guidelines to ensure that surveillance, prevention, and control activities were appropriate for WN. From New York, WN virus spread rapidly westward and by 2002 had been detected in 44 of the United States, including Washington State west of the Continental Divide. The incursion of WN into California seems imminent. In addition to WN virus, California is vulnerable to introduction of other highly virulent mosquito-borne viruses, such as Japanese encephalitis, dengue, yellow fever, Rift Valley fever, and Venezuelan encephalitis viruses. If an existing or introduced virus is detected, it is critical that local and state agencies are prepared to respond in a concerted effort to protect people and animals from infection and disease. The current document describes an enhanced surveillance and response program for mosquito-borne viruses in the State of California. Its contents represent the collective effort of the California Department of Health Services (DHS), the Mosquito and Vector Control Association of California (MVCAC), and the University of California at Davis (UCD) and Berkeley (UCB).

Background

Mosquito-borne viruses belong to a group of viruses commonly referred to as arboviruses (for **ar**thropod-**bo**rne). Although 12 mosquito-borne viruses are known to occur in California, only western equine encephalomyelitis virus (WEE) and St. Louis encephalitis virus (SLE) are significant causes of human disease. WN is anticipated to have a serious impact upon the health of humans, horses, wild birds, and zoo collections when it invades California. Consequently, the California Arbovirus Surveillance Program emphasizes forecasting and monitoring the temporal and spatial activity of WEE, SLE, and WN. These viruses are maintained in nature in wild bird-mosquito cycles that do not depend upon infections of humans or domestic animals to persist. Surveillance and control activities focus on this maintenance cycle, which involves primarily the western encephalitis mosquito, *Culex tarsalis*, and birds such as house finches and sparrows.

Immature stages (called larvae and pupae) of *Culex tarsalis* can be found throughout California in a wide variety of aquatic sources, ranging from clean to highly polluted waters. Most such water is associated with irrigation of agricultural crops or urban wastewater. Other mosquito species, such as *Culex pipiens* and *quinquefasciatus*, may play an important role in SLE and WN transmission cycles in urban and suburban areas. *Ochlerotatus melanimon*, a floodwater mosquito, plays a role in a secondary transmission cycle of WEE involving rabbits. Additional mosquitoes such as *Aedes vexans* and *Culex erythrothorax* could be important bridge (i.e. bird to mammal) vectors in WN transmission.

Mosquito control is the only practical method of protecting people and animals. There are no known specific treatments or cures for diseases caused by these viruses. Vaccines are not available for public use. Infection by WEE virus tends to be most serious in very young children, whereas infection caused by SLE and WN viruses affects elderly people most seriously. WEE and WN can be an important disease in horses and emus, and WN kills a wide variety of endemic and imported birds. There are WEE and WN vaccines available to protect horses.

Mosquito-borne disease prevention strategies must be based on a well-planned, area-wide integrated pest management (IPM) based program. The primary components of an IPM program include education, surveillance, and mosquito control.

Education

Residents, farmers, and duck club owners can play an important role in reducing the number of adult mosquitoes by eliminating standing water that may support the development of immature mosquitoes. For instance, residents can help by properly disposing of discarded tires, cans, or buckets; emptying plastic or unused swimming pools; and unclogging blocked rain gutters around homes or businesses. Farmers and ranchers can be instructed to use irrigation practices that do not allow water to stand for extended periods, and duck club owners can work with mosquito control agencies to determine optimum flooding schedules. Educating the general public regarding curtailing outdoor activities during peak mosquito biting times, using insect repellents, and wearing long-sleeved clothing will help reduce exposure to mosquitoes. Clinical surveillance is enhanced through education of the medical community to recognize the symptoms of WEE, SLE, and WN and to request appropriate laboratory tests. Public health officials need to be alerted if a mosquito-borne viral disease is detected, especially if the public health risk is high.

Surveillance

Surveillance includes the monitoring of climatic factors, estimating immature and adult mosquito abundance, and assessing virus activity by testing mosquitoes, sentinel chickens and wild birds (including dead birds for WN), horses, and humans for evidence of infection. Surveillance must focus not only on mosquito-borne viruses known to exist in California, but be sufficiently broad to also detect newly introduced viruses.

Mosquito Abundance

Mosquito abundance can be estimated through collection of immature or adult mosquitoes. The immature stages (larvae and pupae) can be collected from water sources where mosquitoes lay their eggs. A long-handled ladle (“dipper”) is used to collect water samples and the number of immature mosquitoes per "dip" estimated. In most local mosquito control agencies, technicians search for new sources and inspect known habitats for mosquitoes on a 7 to 14-day cycle. These data are used to direct control operations. Maintaining careful records of immature mosquito occurrence, developmental stages treated, source size, and control effectiveness can provide an early warning to forecast the size of the adult population.

Adult mosquito abundance is a key factor contributing to the risk of disease transmission. Monitoring the abundance of adult mosquito populations provides important information on the size of the vector population as it responds to changing climatic factors and on the effectiveness of larval control efforts. Four adult mosquito sampling methods are currently used in California: New Jersey light traps, carbon dioxide-baited traps, gravid (egg-laying) traps, and resting adult mosquito collections. The advantages and disadvantages of these sampling methods, and guidelines for the design, operation, and processing of the traps have been discussed in the recently published Guidelines for Integrated Mosquito Surveillance (Meyer et al. 2003) and are summarized in Appendix A.

Mosquito Infections

Early detection of virus activity may be accomplished by testing adult mosquitoes for virus infection. Because *Culex tarsalis* is the primary amplifying vector of WEE and SLE and most likely WN, surveillance efforts emphasize the testing of this species. Other species that should be tested include the *Culex pipiens* complex and *Ochlerotatus melanimon.* Female mosquitoes are trapped, usually using carbon dioxide-baited or gravid traps, and pooled into groups of 50 females each for testing at the Davis Arbovirus Research Unit (DARU) laboratory, a part of the UC Davis Center for Vector-borne Disease Research. Procedures for processing mosquitoes for virus infection are detailed in Appendix B. The current surveillance system is designed to detect WN and other vector-borne viruses, in addition to SLE and WEE. Although generally less sensitive than sentinel chickens, mosquito infections may be detected earlier in the season than chicken seroconversions and therefore provide an early warning of virus activity. Testing adult mosquitoes for infection is one of the best methods to detect newly introduced mosquito-borne viruses that would not otherwise be expected to be present in the state. Sampling mosquito species other than *Cx. tarsalis* may be necessary to detect the introduction of viruses that do not have a primary avian-*Culex* transmission cycle.

Avian Infections

Detection of transmission of arboviruses in bird populations can be accomplished by using caged chickens as sentinels and bleeding them routinely to detect viral antibodies (seroconversions), by collecting and bleeding wild birds to detect viral antibodies, or by necropsy of dead birds and testing for WN virus. In California, flocks of ten chickens are placed in locations where mosquito abundance is known to be high or where there is a history of virus activity. Each chicken is bled every two weeks by pricking the comb and collecting blood on a filter paper strip. The blood is tested at DHS' Viral and Rickettsial Disease Laboratory for antibodies to SLE, WEE, and WN. Some agencies conduct their own testing, but send positive samples to DHS for confirmation and official reporting. Because SLE cross-reacts with WN in antibody testing, serum drawn from SLE or WN positive chickens are confirmed at DARU by cross neutralization tests. Frequent testing of strategically placed flocks of sentinel chickens provides the most sensitive and cost-effective method to monitor encephalitis virus activity. Because chickens are continuously available to host-seeking mosquitoes, they are usually exposed to more mosquitoes than can be collected by trapping, especially when adult mosquito abundance is low. Sentinel housing, bleeding instructions, and testing protocols are provided in Appendix C.

Virus activity in wild bird populations can be monitored by bleeding young (hatching year) birds to detect initial virus infection or by bleeding after hatching year birds to determine if the prevalence of the virus in the region has changed. New infection can be detected in recaptured banded birds. In contrast to the convenience of using sentinel chickens, the repeated collection and bleeding of wild birds generally is too labor intensive, technically difficult, and expensive for local mosquito control agencies to perform routinely. In addition, the actual place where a wild bird became infected is rarely known, because birds usually are collected during daylight foraging flights and not at nocturnal roosting sites where they are most frequently bitten by mosquitoes.

Unlike the endemic encephalitides, WN virus frequently causes death in North American birds, especially those in the family Corvidae (e.g. crows, magpies, jays). In 2000, surveillance for WN virus in dead birds was initiated because of its demonstrated utility of providing an early indication that WN is present and that there is a high risk for human infection within the region.

Although there currently is no evidence of the presence of WN in California, WN could be imported to California through interstate or international transport of birds, mosquitoes, or mammals. Another possible source for introduction is by interchange of infected birds between the Atlantic, Mississippi, and Pacific flyways. In collaboration with many local, state, and federal agencies, birds that meet certain criteria are being tested for WN. The communication and testing algorithm for the dead bird surveillance program is detailed in Appendix D.

Equine Infections

Currently, equine disease due to WEE is not a sensitive indicator of epizootic (the occurrence of infections in animals other than humans) activity in California because of the widespread vaccination of equines (horses, donkeys, and mules) against WEE virus. A similar scenario may unfold for WN virus as horse owners begin vaccinating to protect their horses. If confirmed cases do occur, it is a strong indication that WEE or WN is active in that region of the State. Veterinarians are contacted annually by DHS and the California Department of Agriculture (CDFA) to ensure that equines are vaccinated and to describe diagnostic services that are available in the event of a suspected case of WEE or WN encephalitis. Besides WEE and WN, other mosquito-borne viruses may also cause encephalitis in horses, and consequently, testing of equine specimens has been expanded to include other viruses (see Appendix E).

Human Infections

Local mosquito control agencies rely on the rapid detection and reporting of confirmed human cases to plan and implement emergency control activities to prevent additional infections. However, human cases of arboviral infection are an insensitive surveillance indicator of virus activity, because most human infections cause no, or only mild, symptoms. The focus of human WNV, SLE and WEE surveillance is on severe cases, typically encephalitis in any age group or aseptic meningitis in adults. Since transmission may occur from blood or transplanted organs, blood banks and organ transplantation programs have begun screening procedures. In an attempt to stimulate detection of human SLE, WEE, and WN cases in California, communication with key hospitals and local health officials has been enhanced. Specimens from suspect cases entered in DHS' California Encephalitis Project are tested for 15 core agents--including SLE, WEE, and WN. For patients with extensive mosquito exposure in which SLE, WEE, and WN are negative, other arboviruses are added to the core panel. Many local health departments as well as private laboratories now have the capability to conduct screening testing for WNV. Positive specimens can be submitted to the VRDL for confirmation. Physicians are required to report viral encephalitis and viral meningitis cases to their local health department. Laboratories are required to report cases of arboviral encephalitis (Title 17 Sections 2500 and 2505). Cases that are confirmed to be due to WN, SLE or WEE will be investigated by local or state health officials to determine if the infection was acquired locally, imported from a region outside the patient's residence, or acquired by a non-mosquito route of exposure such as blood transfusion, organ donation or previously unidentified exposure sources. Appendix F contains the protocol for submission of laboratory specimens for human disease and Appendix G provides the surveillance case definition for confirmed WN virus infection in humans.

Mosquito Control

Mosquito control is the only practical method of protecting people and animals from mosquito-borne diseases. Mosquito control in California is conducted by over 70 local agencies, including mosquito and vector control districts, environmental health departments, and county health

departments. Compounds currently approved for larval and adult mosquito control in California are listed in Appendix H.

Larval Control

Control of mosquito larvae and pupae prevents mosquitoes from becoming biting female adults capable of transmitting disease, causing discomfort, and ultimately producing another generation of mosquitoes. Larval control focuses target-specific agents in definable aquatic breeding sites. For these reasons, most mosquito control agencies in California target the immature stages rather than the adult stage of the mosquito. Larval mosquito control has three key components: environmental management, biological control, and chemical control.

Environmental management decreases habitat availability or suitability for immature mosquitoes. Environmental management may include water management, such as increasing the water disposal rate through evaporation, percolation, recirculation, or drainage. Controlled irrigation or the careful timing of wetland flooding for waterfowl can reduce mosquito production. Environmental management also may entail vegetation management because emergent vegetation provides food and refuge for mosquito larvae. Management strategies include the periodic removal or thinning of vegetation, restricting growth of vegetation, and controlling algal growth.

Biological control uses natural predators, parasites, or pathogens to reduce immature mosquito numbers. Mosquitofish, *Gambusia affinis*, are the most widely used biological control agent in California. These fish are released annually in a variety of habitats, such as rice fields, small ponds, and canals.

There are several mosquito control products that are highly specific and thus have minimal impact on non-target organisms. These include microbial control agents, such as *Bacillus thuringiensis israelensis* (Bti) and *Bacillus sphaericus*. Insect growth regulators, such as methoprene, prevent immature mosquitoes from developing into adults. Surface films are very effective against both larvae and pupae, but also may suffocate other surface breathing aquatic insects. Organophosphate pesticides are used infrequently because of their impact on nontarget organisms and the environment.

Adult Control

When larval control is not possible or has been used to the fullest extent possible, adult mosquito control may be required to suppress populations of infected mosquitoes and stem an epidemic. Adult mosquito control products may be applied using ground-based equipment, fixed wing airplanes, or helicopters. These products include organophosphates, such as malathion and naled, and pyrethroids, such as resmethrin, sumithrin, and permethrin.

Factors to consider when selecting a pesticide include: 1) efficacy against the target species or life cycle stage, 2) pesticide resistance, 3) pesticide label requirements, 4) availability of pesticide and application equipment, 5) environmental conditions, 6) cost, and 7) toxicity to nontarget species, including humans.

Response Levels

The California Mosquito-borne Virus Surveillance and Response Plan was developed to provide a semi-quantitative measure of virus transmission risk that could be used by local agencies to plan and modulate control activities. Independent models are presented for WEE, SLE, and WN to accommodate the different ecological dynamics of the three viruses (Barker et al. 2003). Six to eight surveillance factors are analyzed to determine the potential for virus transmission and thereby gauge the appropriate response level:

1. Environmental conditions (snowpack, rainfall, temperature, season)
2. Adult mosquito vector abundance
3. Virus isolations from mosquitoes
4. Sentinel chicken seroconversions
5. Fatal infections in birds
6. Infections in equids and ratites
7. Infections in humans
8. Proximity of detected virus activity to urban or suburban regions

Each factor is scored on an ordinal scale from 1 (least severe) to 5 (most severe). The mean score calculated from these factors corresponds to a response level as follows: normal season (1.0 to 2.5), emergency planning (2.6 to 4.0), and epidemic (4.1 to 5.0). Table 1 provides a worksheet to assist in determining the appropriate rating for each of the risk factors for each of the three viruses. Appendix I shows sources of data useful in the calculation of risk in Table 1. The term "average" refers to averages over non-epidemic years in a specific region, such as that within the boundaries of a local mosquito and vector control district. Averages typically are determined for the preceding five-year period (perhaps longer for environmental variables). The ratings listed in Table 1 are benchmarks only and may be modified as appropriate to the conditions in each specific region or biome of the state. Roles and responsibilities of key agencies involved in carrying-out the surveillance and response plan are outlined in "Key Agency Responsibilities."

Each of these surveillance factors can differ in impact and significance according to time of year and geographic region. Climatic factors provide the earliest indication of the potential for virus transmission and constitute the only risk factor actually measured from the start of the calendar year through mid-spring when enzootic surveillance commences in most areas. Other biological factors that emerge as the season progresses are typically, in order: mosquito abundance, infections in non-humans (e.g., mosquitoes, sentinel chickens, equids), and infections in humans.

Each of the three viruses differs in its response to ecological conditions. WEE activity typically is greatest during El Niño conditions of wet winters, excessive run-off, cool springs, increased *Cx. tarsalis* abundance, and virus spillover into *Ochlerotatus* populations. In contrast, SLE activity appears to be greatest during La Niña conditions of drought and hot summer temperatures. Because equine infections with SLE do not result in disease, equine cases are not included in the SLE risk assessment. The SLE response to climatic factors serves as a proxy for WN until further research elucidates the ecology of this virus in California. Abundance and infection of the *Culex pipiens* complex are included in both SLE and WN estimates of risk because of possible transmission by this mosquito in urban environments. The occurrence of dead bird infections is included as a risk factor in the WN calculations.

Table 1. Mosquito-borne Virus Risk Assessment

WEE Surveillance Factor	Assessment Value	Benchmark	Assigned Value
1. **Environmental Conditions** Favorable environmental conditions include above normal rainfall, snow pack, and runoff and cool early season ambient temperature followed by a strong warming trend (El Niño season).	1	Cumulative rainfall and runoff well below average	
	2	Cumulative rainfall and runoff below average	
	3	Cumulative rainfall and runoff average	
	4	Cumulative rainfall and runoff above average	
	5	Cumulative rainfall and runoff well above average	
2**. Adult *Culex tarsalis and Ochlerotatus melanimon*** (bridge vector) **abundance** Determined by trapping adults, identifying them to species, and comparing numbers to averages previously documented for an area.	1	*Cx. tarsalis* abundance well below average (<50%)	
	2	*Cx. tarsalis* abundance below average (50-90%)	
	3	*Cx. tarsalis* abundance average (90-150%)	
	4	*Cx. tarsalis* and *Oc. melanimon* abundance above average (150-300%)	
	5	*Cx. tarsalis and Oc. melanimon* abundance well above average (>300%)	
3. **Virus isolation rate in *Cx. tarsalis and Oc. melanimon* mosquitoes** Tested in pools of 50. Test results expressed as minimum infection rate (MIR) per 1,000 female mosquitoes tested (or per 20 pools).	1	*Cx. tarsalis* MIR / 1000 = 0	
	2	*Cx. tarsalis* MIR / 1000 = 0–1.0	
	3	*Cx. tarsalis* MIR / 1000 = 1.1-2.0	
	4	*Cx. tarsalis* MIR / 1000 = 2.1-5.0 and/or *Oc. melanimon* MIR/1000 > 0	
	5	*Cx. tarsalis* MIR / 1000 > 5.0 and *Oc. melanimon* MIR/1000 >0	
4. **Sentinel chicken seroconversion** Number of chickens in a flock that develop antibodies to WEE virus. If more than one flock is present in a region, number of flocks with seropositive chickens is an additional consideration. Typically 10 chickens / flock.	1	No seroconversions	
	2	One seroconversion in single flock over broad area	
	3	One seroconversion in multiple flocks or multiple seroconversions in a single flock in region	
	4	Two to three seroconversions per flock in multiple flocks in region	
	5	More than three seroconversions per flock in multiple flocks in region	
5. **Infections in equines or ratites**	1	No cases	
	3	One case in broad region	
	4	One or two cases in specific region	
	5	More than two cases in specific region	
6. **Human cases**	1	No human cases	
	3	One human case statewide (but not within local jurisdiction or region)	
	5	One or more human cases in region	
7. **Proximity to urban or suburban regions** (score only if virus activity detected) Risk of outbreak is highest in urban areas because of high likelihood of contact between humans and vectors.	1	Virus activity in remote area	
	2	Virus activity in rural areas	
	3	Virus activity in small towns	
	4	Virus activity in suburban areas	
	5	Virus activity in urban area	
<u>Response Level / Average Rating:</u> Normal Season (1.0 to 2.5) Emergency Planning (2.6 to 4.0) Epidemic (4.1 to 5.0)		**TOTAL**	
		AVERAGE	

SLE Surveillance Factor	Assessment Value	Benchmark	Assigned Value
1. **Environmental Conditions** Favorable environmental conditions include above normal temperatures with or without above normal water conditions of rainfall, snow pack, and runoff. Urban mosquitoes breeding in municipal water systems may benefit from below normal rainfall. (La Niña season)	1	Temperature well below average	
	2	Temperature below average	
	3	Temperature average	
	4	Temperature above average	
	5	Temperature well above average	
2. **Adult *Culex tarsalis or pipiens complex* abundance** Determined by trapping adults, identifying them to species, and comparing numbers to those previously documented for an area.	1	Vector abundance well below average (<50%)	
	2	Vector abundance below average (50-90%)	
	3	Vector abundance average (90-150%)	
	4	Vector abundance above average (150-300%)	
	5	Vector abundance well above average (>300%)	
3. **Virus isolation rate in *Culex tarsalis and Cx. pipiens complex* mosquitoes** Tested in pools of 50. Test results expressed as minimum infection rate (MIR) per 1,000 female mosquitoes tested (or per 20 pools).	1	MIR / 1000 = 0	
	2	MIR / 1000 = 0-1.0	
	3	MIR / 1000 = 1.1-2.0	
	4	MIR / 1000 = 2.1-5.0	
	5	MIR / 1000 > 5.0	
4. **Sentinel chicken seroconversion** Number of chickens in a flock that develop antibodies to SLE virus. If more than one flock is present in a region, number of flocks with seropositive chickens is an additional consideration. Typically 10 chickens / flock.	1	No seroconversions	
	2	One seroconversion in single flock over broad area	
	3	One seroconversion in multiple flocks in region	
	4	Two to three seroconversions per flock in multiple flocks in region	
	5	More than three seroconversions per flock in multiple flocks in region	
5. **Human cases**	1	No human cases	
	3	One human case statewide (but not within local jurisdiction or region)	
	5	One or more human cases in region	
6. **Proximity to urban or suburban regions** (score only if virus activity detected) Risk of outbreak is highest in urban areas because of high likelihood of contact between humans and vectors.	1	Virus activity in remote area	
	2	Virus activity in rural areas	
	3	Virus activity in small towns	
	4	Virus activity in suburban areas	
	5	Virus activity in urban area	
<u>Response Level / Average Rating:</u> Normal Season (1.0 to 2.5) Emergency Planning (2.6 to 4.0) Epidemic (4.1 to 5.0)		**TOTAL**	
		AVERAGE	

WN Surveillance Factor	Assessment Value	Benchmark	Assigned Value
1. **Environmental Conditions** Favorable environmental conditions in California unknown. Rural transmission may favor El Niño conditions, whereas urban transmission may favor La Niña conditions.	1	Temperature well below average	
	2	Temperature below average	
	3	Temperature average	
	4	Temperature above average	
	5	Temperature well above average	
2. **Adult *Culex tarsalis and Cx. pipiens complex* abundance** Determined by trapping adults, identifying them to species, and comparing numbers to those previously documented for an area.	1	Vector abundance well below average (<50%)	
	2	Vector abundance below average (50-90%)	
	3	Vector abundance average (90-150%)	
	4	Vector abundance above average (150-300%)	
	5	Vector abundance well above average (>300%)	
3. **Virus isolation rate in *Culex tarsalis* and *Cx. pipiens* complex mosquitoes** Tested in pools of 50. Test results expressed as minimum infection rate (MIR) per 1,000 female mosquitoes tested (or per 20 pools).	1	MIR / 1000 = 0	
	2	MIR / 1000 = 0-1.0	
	3	MIR / 1000 = 1.1-2.0	
	4	MIR / 1000 = 2.1-5.0	
	5	MIR / 1000 > 5.0	
4. **Sentinel chicken seroconversion** Number of chickens in a flock that develop antibodies to WN virus. If more than one flock is present in a region, number of flocks with seropositive chickens is an additional consideration.	1	No seroconversions in California	
	2	Seroconversion in neighboring state, but not CA	
	3	One seroconversion in single flock over broad area	
	4	One seroconversion in one or more flocks in region	
	5	Two or more seroconversions per flock in one or more flocks in region	
5. **Dead bird infection** Includes zoo collections.	1	No WN positive dead birds in California	
	2	WN positive dead bird in neighboring state, but not CA	
	3	One confirmed WN positive dead bird in California, but none in specific region	
	4	One confirmed WN positive dead bird reported in specific region	
	5	Multiple confirmed WN positive dead birds and multiple reports of dead birds in region	
6. **Equine cases**	1	No equine cases	
	3	One equine case in broad region	
	4	One equine case in specific region	
	5	Multiple equine cases in specific region	
7. **Human cases**	1	No human cases	
	3	One human case statewide (but not within specific region)	
	4	One human case in specific region	
	5	Multiple human cases in specific region	
8. **Proximity to urban or suburban regions** (score only if virus activity detected) Risk of outbreak is highest in urban areas because of high likelihood of contact between humans and vectors.	1	Virus activity in remote area	
	2	Virus activity in rural areas	
	3	Virus activity in small towns	
	4	Virus activity in suburban areas	
	5	Virus activity in urban area	
<u>Response Level / Average Rating:</u> Normal Season (1.0 to 2.5) Emergency Planning (2.6 to 4.0) Epidemic (4.1 to 5.0)		**TOTAL**	
		AVERAGE	

Characterization of Conditions and Responses

Level 1: Normal Season

Risk rating: 1.0 to 2.5

CONDITIONS
• Average or below average snowpack and rainfall; average seasonal temperatures • Mosquito abundance at or below five year average (key indicator = adults of vector species) • No virus isolations from mosquitoes • No seroconversions in sentinel chickens • No WN infected dead birds • No equine cases • No human cases
RESPONSE
• Conduct routine public education (eliminate standing water around homes, use personal protection measures) • Conduct routine mosquito and virus surveillance activities • Conduct routine mosquito larval control • Inventory pesticides and equipment • Evaluate pesticide resistance in vector species • Ensure adequate emergency funding • Release routine press notices • Send routine notifications to physicians and veterinarians • Establish and maintain routine communication with local office of emergency services personnel; obtain Standardized Emergency Management System (SEMS) training

Level 2: Emergency Planning

Risk rating: 2.6 to 4.0

CONDITIONS
• Snowpack and rainfall and/or temperature above average • Adult mosquito abundance greater than 5-year average (150% to 300%) • One or more virus isolations from mosquitoes (MIR / 1000 is <5) • One to three chicken seroconversions per flock of 10 birds • One WN positive dead bird in California or in specific region • One or two equine cases statewide • One human case statewide • Viral activity in small towns or suburban area
RESPONSE
• Review epidemic response plan • Enhance public education (include messages on the signs and symptoms of encephalitis; seek medical care if needed; inform public about pesticide applications if appropriate) • Enhance information to public health providers • Conduct epidemiological investigations of cases of equine or human disease • Increase surveillance and control of mosquito larvae • Increase adult mosquito surveillance • Increase number of mosquito pools tested for virus • Conduct localized chemical control of adult mosquitoes • Contact commercial applicators in anticipation of large scale adulticiding • Review candidate pesticides for availability and susceptibility of vector mosquito species • Ensure notification of key agencies of presence of viral activity, including the local office of emergency services

Level 3: Epidemic Conditions

Risk rating: 4.1 to 5.0

CONDITIONS
• Snowpack, rainfall, and water release rates from flood control dams and/or temperature well above average • Adult vector population extremely high (>300%) • Virus isolates from multiple pools of mosquitoes (MIR / 1000 > 5.0) • More than three seroconversions per flock of ten birds in multiple flocks • Multiple confirmed WN positive dead birds and multiple reports of dead birds in region • More than two equine cases in specific region • One or more human cases in region • Virus detection in urban or suburban areas
RESPONSE
• Conduct full scale media campaign • Alert physicians and veterinarians • Conduct active human case detection • Conduct epidemiological investigations of cases of equine or human disease • Continue enhanced larval surveillance and control of immature mosquitoes • Broaden geographic coverage of adult mosquito surveillance • Accelerate adult mosquito control if appropriate • Coordinate the response with the local Office of Emergency Services or if activated, the Emergency Operation Center (EOC) • Initiate mosquito surveillance and control in geographic regions without an organized vector control program • Request public health exemptions from FIFRA (40 CFR 166) and emergency tolerance exemptions (40 CFR 176) • Determine whether declaration of a local emergency should be considered by the County Board of Supervisors (or Local Health Officer) • Determine whether declaration of a “State of Emergency” should be considered by the Governor at the request of designated county or city officials • Ensure state funds and resources are available to assist local agencies at their request • Determine whether to activate a Standardized Emergency Management System (SEMS) plan at the local or state level • Continue mosquito education and control programs until mosquito abundance is substantially reduced and no additional human cases are detected

Key Agency Responsibilities

Local Mosquito and Vector Control Agencies

- Gather, collate, and interpret regional climate and weather data.
- Monitor abundance of immature and adult mosquitoes.
- Collect and submit mosquito pools for virus detection.
- Maintain sentinel chicken flocks, obtain blood samples, and send samples to laboratory.
- Pick-up and ship dead birds for WN testing.
- Conduct routine control of immature mosquitoes.
- Conduct control of adult mosquitoes when needed.
- Educate public on mosquito avoidance and reduction of mosquito breeding sites.
- Coordinate with local Office of Emergency Services personnel.

Mosquito and Vector Control Association of California

- Coordinate purchase of sentinel chickens.
- Receive, track, and disperse payment for surveillance expenses.
- Coordinate surveillance and response activities among member agencies.
- Serves as spokesperson for member agencies.
- Establish liaisons with press and government officials.

California Department of Health Services

- Collate adult mosquito abundance data submitted by local agencies; provide summary of data to local agencies.
- Maintain a WN virus information and dead bird reporting hotline, 1-877-WNV-BIRD, and a WN virus website. http://westnile.ca.gov/
- Coordinate submission of specimens for virus testing.
- Maintain database of all specimens tested.
- Test sentinel chicken sera for viral antibodies.
- Test human specimens for virus.
- Distribute a weekly bulletin summarizing surveillance test results.
- Send weekly surveillance results to the UC Davis interactive website.
- Immediately notify local vector control agency and public health officials when evidence of viral activity is found.
- Conduct epidemiological investigations of cases of equine and human disease.
- Coordinate and participate in a regional emergency response in conjunction with California Office of Emergency Services.
- Conduct active surveillance for human cases.
- Provide oversight to local jurisdictions without defined vector-borne disease control program.
- Maintain inventory of antigens and antisera to detect exotic viruses.

University of California at Davis

- Conduct research on arbovirus surveillance, transmission of mosquito-borne diseases, and mosquito ecology and control.
- Test mosquito pools and dead birds for virus.

- Provide a panel of tests for identification of viruses from human, equine, bird, or arthropod vectors.
- Maintain an interactive website for dissemination of mosquito-borne virus information and data.
- Maintain inventory of antigens, antisera, and viruses to detect the introduction of exotic viruses.
- Provide confirmation of tests done by local or state agencies.

California Department of Food and Agriculture
- Notify veterinarians and veterinary diagnostic laboratories about WEE and WN and testing facilities available at UCD Center for Vector-borne Disease Research.
- Provide outreach to general public and livestock and poultry producers on the monitoring and reporting of equine and ratite encephalitides.
- Facilitate equine and ratite sample submission from the field.

California Animal Health and Food Safety Laboratory
- Identify and screen dead birds for WNV testing.
- Conduct necropsies and testing on dead crows and other birds.
- Submit bird tissues to UCD for testing.

Local Health Departments
- Refer human and equine specimens to DHS or UCD for further testing.
- Notify local medical community, including hospitals and laboratories, if evidence of viral activity present.
- Participate in emergency response.
- Conduct epidemiological investigations of cases of human disease.
- Assist in public education.

Governor's Office of Emergency Services
- Coordinate the local, regional, or statewide emergency response under epidemic conditions in conjunction with DHS via the Standardized Emergency Management System (SEMS).
- Serve as liaison with the Federal Emergency Management Agency (FEMA) in the event that a federal disaster has been declared.

Centers for Disease Control and Prevention
- Provide consultation to state and local agencies in California if epidemic conditions exist.
- Provide national surveillance data to state health departments.

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Appendix A: Guidelines for Adult Mosquito Surveillance

The objective of Appendix A is to standardize adult mosquito sampling and reporting procedures to provide comparable and interpretable surveillance results among collaborating mosquito control agencies in California. This section summarizes information from Guidelines for Integrated Mosquito Surveillance in California that recently has been adopted by the Mosquito and Vector Control Association (MVCAC), (Meyer et al. 2003). The MVCAC approach stratifies the use of different sampling methods in rural, small town, and urban environments for each of the major biomes of California and provides a listing of target vector and nuisance mosquito species. The stratified sampling approach monitors vector populations and virus activity in rural enzootic foci, agricultural, or suburban amplification sites, and densely populated urban centers to provide estimates of early, eminent, and current epidemic risk.

The four sampling methods currently used by mosquito control agencies are: 1) New Jersey (American) light trap, 2) CDC style CO_2-baited trap, 3) gravid trap, and 4) adult resting collections. These guidelines describe: 1) a comparison of the sampling methods, 2) equipment design, 3) operation, 4) specimen processing, 5) data recording and analysis, and 6) data usage.

Advantages and Disadvantages of Mosquito Sampling Methods:

New Jersey Light Trap

Pros	Cons
• All female metabolic states and males collected • Minimal collection effort (can be run nightly without service) • Long history of use in California	• Selective for phototaxic nocturnally active mosquitoes • Ineffective with competing light sources • Sorting time excessive because of other insects in traps • Specimens dead; less use for virus detection • Collects comparatively few specimens

CDC/EVS CO_2 Trap

Pros	Cons
• Samples biting population • Collects large numbers of virus vector species • Specimens alive; suitable for virus detection • Without light, collects mostly mosquitoes thus reducing sorting time • Battery operated, portable	• Collects >50% nullipars (have never oviposited) • Must be set and picked-up daily • Dry ice cost high; availability can be a problem • Does not collect males or blooded and gravid females

Gravid Trap

Pros	Cons
• Collects females that have bloodfed; may have higher infection rate • Specimens alive; suitable for virus detection • Extremely sensitive for *Cx. quinquefasciatus* in urban habitat • Bait inexpensive • Battery operated, portable	• Collects only foul-water *Culex* • Bait has objectionable odor • Must be set and picked-up daily

Resting Catches

Pros	Cons
• All metabolic states collected • Minimal equipment needed • Specimens alive; suitable for virus detection • Blooded and gravid specimens can be tested to improve sensitivity of virus surveillance	• Quantification difficult due to: 1. shelter size and type 2. collector efficiency • Labor intensive

New Jersey (American) Light Trap (NJLT)

Trap specifications and components (Mulhern 1953)

1. Ten inch diameter trap tube of sufficient length to accommodate motor, fan, cone screen, and killing jar. A lockable screen cage or holding strap should be added to the bottom of the trap to prevent tampering with the killing jar.
2. A 4- or 5-bladed 9.0-inch diameter fan.
3. Sealed, heavy-duty type refrigerator motor suspended by three support brackets for added stability; air discharge ≅ 450-500 cu. ft/min.
4. Hood or cone with a two- or three-point chain attachment for trap hanging.
5. One-quarter inch mesh hardware cloth over the mouth of the trap tube to preclude entry by large moths and other debris.
6. Timer or photoelectric eye to turn trap on/off. The photoelectric eye is preferred to prevent disruptions of trapping time that may occur with a timer due to power outages.
7. 25w, 110v, frosted light bulb.
8. Exterior trap color is insignificant, but underside of hood should be painted white to increase light intensity (Barr et al. 1963).
9. Killing jar with warning label containing a dichlorvos "no-pest" strip should be replaced every three months. A pint or quart jar could be used depending on the number of insects caught.

Operation

At a minimum, trap should be located in each principal municipality of a district or have a distribution of one trap/township (36 sq. mi.) to sample the adult mosquito population within the boundaries of the district's responsibility. Correct placement of the NJLT is a critical factor in its performance as a surveillance mechanism for measuring the relative abundance of phototaxic mosquitoes. Place the traps at six-foot height. This can be done by using a metal standard, or by hanging the traps from tree limbs or roof eaves. These distances should maximize attractancy over a 360 degree radius. The trap should be placed on the leeward side of a structure or tree line to decrease the influence of wind on trap catch.

Traps should be kept away from smoke or chemical odors that may be repellent to the mosquitoes. Traps should be away from buildings in which animals are housed and not in the immediate vicinity of sentinel flocks to diminish attractancy competition. Traps should be placed away from street and security lights that may diminish attractancy of the trap bulb.

Traps should be operated from week 13 to week 43 of the calendar year for districts north of the Tehachapi Mountains and all year long for districts south of the Tehachapi. Ideally, the traps should run for four to seven nights before the collection is retrieved (Loomis and Hanks 1959). The trap should be thoroughly cleaned with a brush to remove spider webs or any other debris that may hinder airflow through the trap. A regular cleaning schedule should be maintained during the trapping season to maintain trap efficiency.

Processing

Adult mosquitoes from the NJLT collection should be sorted from the other insects in an enamel pan before being identified and counted at 10x magnification under a dissecting microscope. Counting aliquots or subsamples of all specimen samples should be discouraged, because vector species may comprise only a small fraction of the total mosquito collection.

CDC style CO_2-baited trap

Trap design and components

Currently there are two types of CO_2-baited traps being used in California: CDC trap and the EVS trap (Pfuntner, 1979), which is a modification of the first. Both trap types are baited either with an insulated container holding 1-2 kg of dry ice or with a cylinder containing compressed CO_2 gas with a regulator that releases 0.5 - 1.0 liters/minute. The dry ice container or the carbon dioxide gas cylinder should be properly labeled as to their contents. Both trap styles use a screened collection bag or a modified gallon ice cream carton with tubular surgical stockinet attached to the bottom of the motor housing unit to retain the collected mosquitoes.

The CDC trap uses:

1. A 3.5" diameter plexiglass cylinder housing a 6v DC motor and a 4-blade fan.
2. Rechargeable 6v battery power source.
3. Aluminum rain shield (optional).

The EVS trap:

1. Uses a 5" diameter PVC cylinder housing a 4.5-6.0v DC motor, and a 2-blade fan.
2. Uses three 1.5v D cell batteries in series as a power source.
3. Lacks an aluminum rain shield above the trap housing.

Operation

Carbon dioxide-baited traps can be used for abundance monitoring or capturing mosquitoes for virus testing. A six foot tall standard should be used to standardize trap placement. Trap location should be standardized for population and virus infection rate monitoring.

Knowledge of the host-seeking patterns of the target species is essential in determining CO_2-baited trap placement in the habitat and will enhance the catch size and therefore sampling sensitivity. *Culex tarsalis* primarily bloodfeed on birds and therefore hunt along vegetative borders and tree canopies where birds roost and nest. *Culex erythrothorax* are best collected within wetland areas near dense stands of tules and cattails. In large, open breeding sources such as rice fields, CO_2-baited traps could be hung on standards on the up-wind side of the source for *Cx. tarsalis* and *Anopheles freeborni* collections. *Ochlerotatus (formerly Aedes) melanimon* and *Oc. nigromaculis* are mammal feeders and typically hunt over open fields.

When used to supplement sentinel chickens for arbovirus surveillance, traps should be operated at different locations to enhance geographical coverage and thus surveillance sensitivity. Labor and time constraints determine the extent of sampling. When used to monitor population abundance, traps should be operated weekly or biweekly at the same fixed stations. Temperature, wind speed, wind direction, and rainfall should be recorded because these factors readily affect catch size. The mini-light attracts other phototaxic insects that may hinder sorting and/or damage female mosquitoes in the collection container while repelling members of the *Cx. pipiens* complex. The CO_2-baited trap should not be placed in immediate proximity to the sentinel chicken flock as it will compete with, and therefore lessen, exposure of the sentinel birds, but should be placed within 100-200m radius.

Maintenance of the traps should be performed regularly. Rechargeable 6v batteries should be charged after each night's run and rechargeable 1.5v batteries should be checked on a battery tester to determine the amount of power left to run the trap motors. Rechargeable 1.5v batteries need to be drained completely before being recharged to maintain full power capacity. Alkaline batteries need to be replaced after every use. The motors, fan blades, and interior of the trap housing should be cleaned on a regular basis.

Processing

Mosquitoes collected for arbovirus surveillance should be processed according to the procedures outlined in Appendix B. Ten pools of a species (*Cx. tarsalis*, *Cx. pipiens, Cx. quinquefasciatus, Ochlerotatus melanimon*, and *Oc. dorsalis*,) should be submitted for virus testing from a given geographical location at a given time. Only live mosquitoes should be pooled for virus testing. Dead, dried specimens should be counted and discarded. Only whole specimens should be submitted; avoid including body parts (which may be from other mosquito species) or other Diptera (i.e., *Culicoides*, etc.) in the pool to prevent sample contamination. Avoid freezing specimens before sorting and counting. Mosquitoes collected for population monitoring are killed, identified under a dissecting microscope, and counted.

Reiter/Cummings gravid traps

Trap design and components

The Reiter/Cummings gravid traps consist of a rectangular trap housing with an inlet tube on the bottom and an outlet tube on the side or top. The rectangular housing is provided with legs to stabilize the trap over the attractant basin containing the hay-infusion mixture. The trap housing contains the motor assembly and collection chamber for gravid mosquitoes. The revised Reiter gravid trap (Reiter 1987) utilizes a 6v powered motor using three D cell batteries, whereas the Cummings modified gravid trap (Cummings 1992) uses a 9V motor and four D cell batteries. Both can be operated using a 6V gel cell battery. Both traps place the collection chamber on the inlet side of the motor so that the fan blades will not damage collected mosquitoes. The inlet height should be two inches above the surface of the hay-infusion medium to create a proper vortex.

The oviposition attractant consists of a fermented infusion made by mixing Timothy or alfalfa hay, lactalbumen, Brewer's yeast and water. The mixture should sit at room temperature for one to two days to allow fermentation and increase attractancy. New solutions should be made at least biweekly to maintain consistent attractancy.

Operation

The Reiter/Cummings gravid trap is primarily used in suburban and urban residential settings, principally for surveillance of *Culex pipiens* complex populations. The trap is placed on the ground near dense vegetation that serves as resting sites for gravid females. Specimens may be retrieved on a one to three day basis.

Processing

Culex pipiens complex females collected with the gravid trap for arbovirus surveillance should be retrieved daily and the protocol for mosquito pool submission as outlined in Appendix B should be followed. For population monitoring of the *Culex pipiens* complex, collections may

be retrieved every third day. The females are killed, identified and counted before being discarded. Autogenous females may also be attracted to the gravid trap.

Adult resting collections

Trap design and operation

A flashlight and mechanical aspirator can be used to collect adult mosquitoes resting in habitats such as shady alcoves, buildings, culverts, or spaces under bridges. Highest numbers usually are collected at humid sites protected from strong air currents. Adults resting in vegetation may be collected using a mechanical sweeper such as the AFS (Arbovirus Field Station) sweeper (Meyer et al. 1983). For quantification, time spent searching is recorded and abundance expressed as number per person-hour.

Red boxes were developed to standardize collections spatially. Different researchers have used red boxes of varying dimensions. Largest catches are made in semi permanent walk-in red boxes which measure 4' x 4' x 6' (Meyer 1985). Smaller 1' x 1' x 1' foot boxes typically collect fewer specimens, but are readily portable. The entrance of the walk-in red box should be left open, draped with canvas, or closed with a plywood door. The canvas or plywood door should have a 1 or 2 ft gap at the bottom to allow entry of mosquitoes, while affording some protection from the wind and decreasing the light intensity within the box. The box entrance should not face eastward into the morning sun or into the predominant wind direction.

Processing

Mosquitoes should be anesthetized, identified under a dissecting microscope, sorted by sex and female metabolic status (i.e., empty or unfed, blood fed or gravid), and counted. Females may be counted into ten pools of approximately 50 females per site per collection date for virus monitoring (see Appendix B). Only living females should be used for arbovirus surveillance. Data on metabolic status may indicate population reproductive age as well as diapause status.

Data recording and analysis

Counts from NJLTs should be recorded on the DHS Adult Mosquito Occurrence Report Summary Form and faxed to (510) 412-6263. For comparisons of abundance over time, space, or collection methods, refer to Biddlingmeyer (1969).

Data usage

Mosquito collections from some or all of the four sampling methods collectively can be used to:

1. Assess control efforts.
2. Compare mosquito abundance from collections with the number of service requests from the public to determine the tolerance of neighborhoods to mosquito abundance.
3. Monitor arbovirus vector abundance and minimum infection rates.
4. Determine proximity of breeding source(s) by the number of males present in collections from the NJLTs and red boxes.
5. Determine age structure of females collected by CO_2 traps and resting adult collections; such data are critical to evaluating the vector potential of the population.

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Appendix B: Procedures for Processing Mosquitoes for Arbovirus Detection

1. Collect mosquitoes alive and return them immediately to the laboratory. Collections should be kept humid during transport with moist toweling to prevent desiccation. Females should be offered 5-10 percent sucrose if held overnight or longer before processing.

2. Anesthetize mosquitoes by cold, carbon dioxide, or triethylamine (TEA). TEA is recommended because specimens are permanently immobilized with minimal mortality and with no loss of SLE or WEE virus titer (Kramer et al. 1990). TEA should be used either outdoors or under a chemical hood. Collections can be knocked down outdoors using a few drops of TEA, the specimens transferred to Petri dishes, and then taken into the laboratory for processing. If refrigerated and kept humid, mosquitoes will remain alive in covered Petri dishes for one or two days without additional anesthesia.

3. Sort mosquito collections to species under a dissecting microscope at 10X to ensure correct identification and to make sure that extraneous mosquito parts (i.e., legs, wings) or other small insects such as chironomids or *Culicoides* are not inadvertently included in the pools. This will be extremely important as diagnostics transition from virus isolation to sensitive RNA methods of viral detection. Count and discard dead and dried mosquitoes. Lots of 50 females (minimum of 12 females) per pool of each vector species from each collection site are then counted. Place each mosquito pool in individual screw-cap cryovials fitted with O-rings to prevent contact with CO_2 during transport and storage. Recommended sampling effort is ten pools of 50 females of each species from each site per week to detect minimum infection rates (MIRs) ranging from 0 to 20 per 1,000 females tested. Vials with pools should be labeled sequentially starting with #1 each year after the site code; e.g., KERN-1-03; where 03 refers to year 2003. VERY IMPORTANT: POOLS MUST BE ACCOMPANIED BY "MOSQUITO POOLS SUBMITTED FORM MBVS-3" AND CAN ONLY BE TESTED FROM REGISTERED SITES (USE FORM MBVS-1 TO REGISTER COLLECTION SITES - see Appendix C).

   List the site code for each pool that consists of a designated four-letter agency code followed by four digits identifying the site, i.e., KERN0001. Keep the pool numbers in sequence for the whole year regardless of the number of site codes, i.e., pool #1 may be from KERN0001, and pool #2 may be from KERN0004.

4. Freeze pools immediately at -70°C either with dry ice in an insulated container or in an ultralow temperature freezer. Pools are shipped frozen on dry ice to the UC Davis Arbovirus Research Unit for testing by an *in situ* enzyme linked immunosorbent assay (EIA). Care must be taken not to allow pools to defrost during storage or shipment, because each thaw and freeze kills approximately half the virus, and all virus will be lost if the specimens sit at room temperature for extended periods. Address shipment to: Davis Arbovirus Research Unit, University of California, Old Davis Road, Davis CA 95616.

Reference: Kramer, L.D., S.B. Presser, E.J. Houk and J.L. Hardy. 1990. Effect of the anesthetizing agent triethylamine on western equine encephalomyelitis and St. Louis encephalitis viral titers in mosquitoes (Diptera: Culicidae). J. Med. Entomol. 27:1008-1010.

MOSQUITO POOLS SUBMITTED TO UCD - 2003

DATE RECD. BY UCD _____

AGENCY CODE: ___ ___ ___ ___ AGENCY

DATE TESTED _____

_____

BULLETIN NO. _____

Point of Contact _____ **Telephone Number** _____

E-mail Address _____

SITE CODE	POOL NO.	SITE NAME	DATE	TRAP TYPE	MOSQ. SPECIES	# IN POOL	WEE	SLE	CE

Form MBVS-3 (Revised 12/19/2002)

Appendix C: Procedures for Maintaining and Bleeding Sentinel Chickens

1. Procure hens in March or April when 18 weeks of age to ensure minimal mortality during handling. Hens at this age have not yet begun to lay eggs, but should have received all their vaccinations and been dewormed.
2. Ten sentinel chickens can be housed in a 3Wx6Lx3H ft coop framed with 2x2 and 2x4 inch construction lumber and screened with 1x1 inch welded wire. The site for each coop must first be registered using FORM MBVS-2 submitted to UC Davis. Coops should be at least two feet off the ground to reduce predator access, facilitate capture of the birds for bleeding, and allow the free passage of the feces through the wire floor to the ground. A single, hinged door should be placed in the middle of the coop, so that the entire coop is accessible during chicken capture. After construction, the lumber and roof should be protected with water seal. A self-filling watering device should be fitted to one end of the coop and a 25 lb. feeder suspended in the center for easy access. In exchange for the eggs, a local person (usually the home owner, farm manager, etc.) should check the birds (especially the watering device) and remove the eggs daily. If hung so the bottom is about four inches above the cage floor and adjusted properly, the feeder should only have to be refilled weekly (i.e., 100 lb. of feed per month per flock of ten birds). Therefore, if proper arrangements can be made and an empty 55 gallon drum provided to store extra feed, sentinel flocks need only be visited biweekly when blood samples are collected.
3. Band each bird in the web of the wing using metal hog ear tags and appropriate pliers. This band number, the date, and site registration number must accompany each blood sample sent to the laboratory for testing.
4. Bleed each hen from the distal portion of the comb using a standard lancet used for human finger "prick" blood samples. The bird can be immobilized by wedging the wings between the bleeder's forearm and thigh, thereby leaving the hand free to hold the head by grabbing the base of the comb with the thumb and forefinger. The comb should be "pricked" with the lancet and blood allowed to flow from the "wound" to form a drop. Collect the blood by touching the end of the pre-numbered filter paper strip (opposite from the number) to the wound. Collect several drops in this fashion to completely soak a pre-marked 3/4 inch long portion of the 3/8 inch wide filter paper strip. Place the numbered end of the strip into the slot of the holder (or "jaws" of the clothes pin) leaving the blood soaked end exposed to air dry.
5. Staple the completely dry filter paper strips through the number along the top end of a 5x8 inch card, leaving the blood soaked end free so that the laboratory staff can readily remove a standard punch sample. Write the name of the flock and the date onto the card and place it and a single flock specific data sheet into a zip lock plastic bag. It is important that blooded ends do not become dirty, wet, or touch each other. VERY IMPORTANT: CHICKEN SERA MUST BE ACCOMPANIED BY SENTINEL CHICKEN BLOOD FORM (MBVS-2) OUTSIDE THE ZIP-LOCK BAG. Samples from each bleeding date then can be placed into a mailing envelope and sent to:

   Department of Health Services, Richmond Campus
   Specimen Receiving Unit Room B106 (ATTN: ARBO)
   850 Marina Bay Parkway
   Richmond, CA 94804

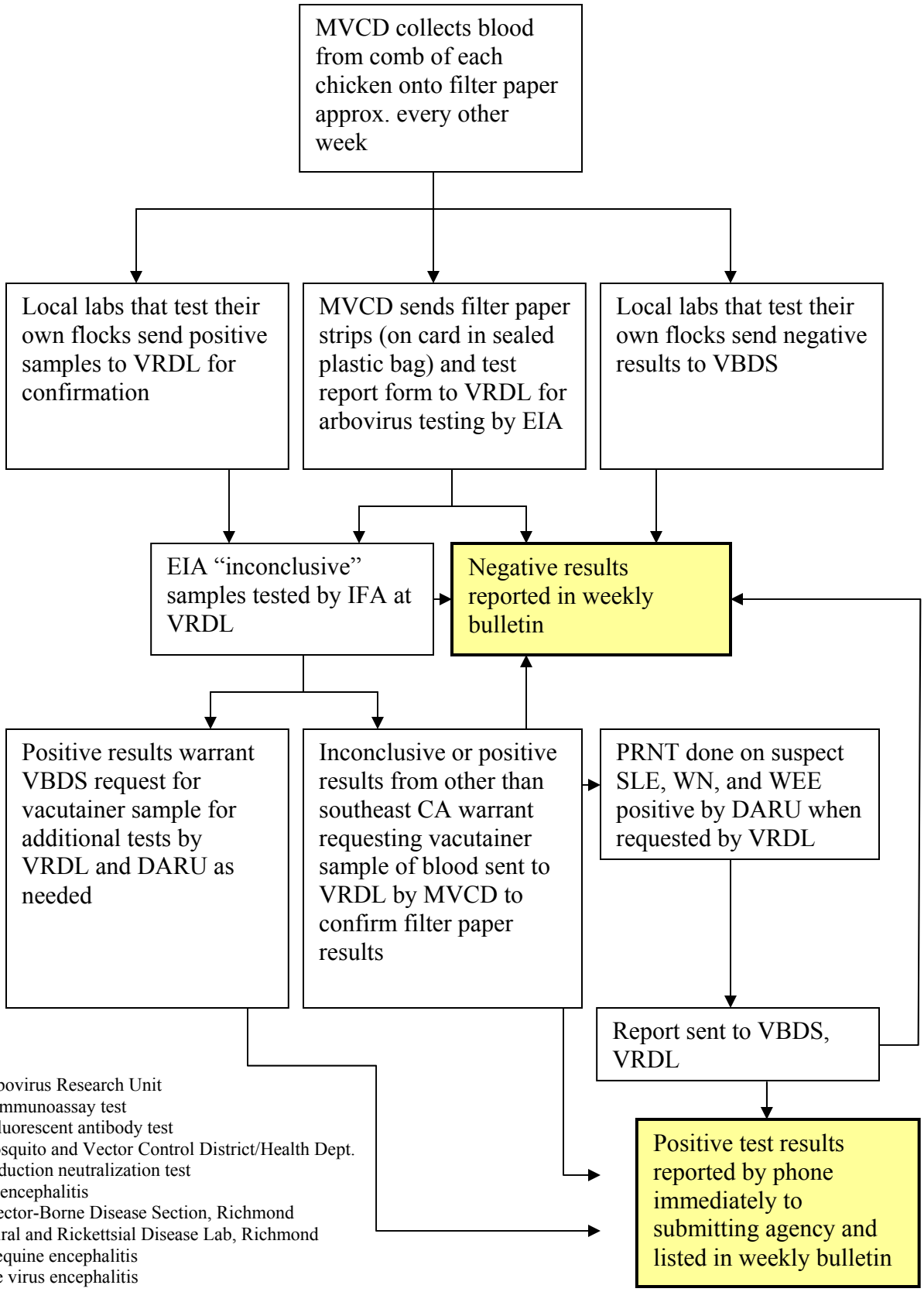
Specimens should be mailed to arrive by Friday afternoon for testing to start the following Monday.

6. In the laboratory, a single punch is removed from the blooded end of the paper and placed into one well of a 96-well plate with 200 µl of diluent. Specimens are allowed to soak overnight and the eluate tested for WEE, SLE, and WN IgG antibody using ELISA. Positive specimens are confirmed the following day using an indirect fluorescent antibody test. SLE or WN positives are sent to DARU for confirmation by cross-neutralization tests.

Reference

Reisen, W.K. 1995. Guidelines for Surveillance and Control of Arboviral Encephalitis in California, In: Interagency Guidelines for the Surveillance and Control of Selected Vector-borne Pathogens in California, Mosquito and Vector Control Association of California, Sacramento.

California Procedure for Testing Sentinel Chickens for the Presence of Antibodies to Flaviviruses (SLE and WN) and WEE



Key:

DARU: Davis Arbovirus Research Unit
EIA: Enzyme immunoassay test
IFA: Indirect fluorescent antibody test
MVCD: Local Mosquito and Vector Control District/Health Dept.
PRNT: Plaque reduction neutralization test
SLE: St. Louis encephalitis
VBDS: CDHS Vector-Borne Disease Section, Richmond
VRDL: CDHS Viral and Rickettsial Disease Lab, Richmond
WEE: Western equine encephalitis
WN: West Nile virus encephalitis

Surveillance for Mosquito-borne Viruses
Registration of Agencies and Sites

1. Participation of agencies

Agencies interested in participating in the statewide surveillance program for mosquito-borne viruses should place orders through the Mosquito and Vector Control Association (MVCAC) for testing of sentinel chicken blood samples and mosquito pools. MVCAC will bill the agency for the number of samples to be tested, register the agency, assign an agency code, and notify VRDL and UC of the names and codes for each registered agency.

As part of an agreement on coordination of surveillance for mosquito-borne viruses, VRDL will accept and test sentinel chicken blood samples only from those California agencies that have placed orders though MVCAC. UC Davis will accept and test mosquito pools only from those agencies that have placed orders though MVCAC.

2. Registration of sentinel flock sites and wing band numbers

Prior to submitting any sentinel chicken blood samples to VRDL, each agency must register each new flock site with UC Davis using the "SURVEILLANCE SITE REGISTRATION" form MBVS-1 (revised 12/1/2002). Blood samples sent to VRDL must be accompanied by the form "SENTINEL CHICKEN BLOOD – 2003" (MBVS-2, revised 12/9/2002) for each flock site.

Fill out a MBVS-2 form for each site and include a four digit numeric code for the site along with the wing band numbers of chickens placed at that site. Also include the date the chickens were bled. VRDL will cross check the agency and site code numbers before testing the samples.

VRDL will test samples only if they are accompanied by the appropriate 2003 form which includes the registered agency code (assigned by MVCAC), the registered site code (assigned by you), and, for blood samples, the wing band numbers assigned to that site.

3. Registration of mosquito sampling sites

Registration of new sites used for collection of mosquitoes for virus testing may be accomplished by faxing a copy of the "SITE SURVEILLANCE REGISTRATION 2003" form to (530) 754-6360 (UC Davis) or e-mailing it to bfeldridge@ucdavis.edu at the same time the pools are shipped to UC Davis. UC will test the pools provided that adequate information is provided on the "MOSQUITO POOL SUBMISSION" form (MBVS-3, revised 12/19/01), including your agency code, your site code for the site and geographic coordinates. If you are unable to determine the geographic coordinates, please provide a map to UC Davis showing the location of each site and its site code.

The geographic coordinates will be used to generate computer maps that will show all registered sites and test results for each site each week. Also, as part of a collaborative effort, UCD will be generating up-to-date maps from the weekly results for posting at http://vector.ucdavis.edu/.

4. **Questions?** Please contact Al Hom, Vector-Borne Disease Section at (510) 412-6254 or arbovirus@dhs.ca.gov.

SURVEILLANCE SITE REGISTRATION 2003

Please fax to 530-754-6360 or email to bfeldridge@ucdavis.edu

AGENCY CODE: ____ ____ ____ ____
(MUST BE 4 LETTERS, NO NUMBERS)

SITE CODE: ____ ____ ____ ____
(MUST BE 4 NUMBERS, NO LETTERS)

This form is for registration of sites used for sentinel chickens, collection of mosquitoes for virus testing, or for mosquito abundance reports. It can be used for any surveillance-related data where repeated collections are made from the same site during the season

_____ _____ _____

Agency Name | County in which site located | Elevation (feet)

Latitude ____° ____' ____" North (Degrees, minutes, seconds)

Longitude ____° ____' ____" West (Degrees, minutes, seconds)

Site is located _____ miles in a _____Direction from _____
(City, town, village, or other place shown on maps)

NUMERICAL SITE DESIGNATION OR NAME USED BY AGENCY FOR IDENTIFICATION:
(e.g., 234A, or Adore Farms)

SITE DESCRIPTION:

LAND USE (E.G., FOREST, FARMLAND, URBAN, SUBURBAN, ETC.)
_____

HABITAT (E,G., POND, VERNAL POOL, BACK YARD, RIVER BANK, ETC.)
_____

SURROUNDINGS (E.G., SCHOOL, HOMES, COUNTY ROAD, ETC.)
_____

OTHER DESCRIPTION
_____
_____
_____
_____

SITE FUNCTIONS:

This site is used for (check all that apply):

- □ Sentinel chickens
- □ Mosquitoes for virus isolation
- □ Mosquitoes for abundance report
- □ Other (describe)

_____

Trap type used (E.G., NJLT)_____

If composite site, number of traps or other collection methods used _____

Form MBVS-1 (Revised 12/1/02)

SENTINEL CHICKEN BLOOD - 2003 (REV. MARCH)

PLEASE DO NOT PLACE THIS SHEET INSIDE THE ZIPLOCK BAG

VRDL PAGE NUMBER

REGISTERED AGENCY CODE: ___ ___ ___ ___ *SITE CODE ___ ___ ___ ___

Name of Agency: _____

Name of Site: _____Nearest City or Place: _____

County: _____

DATE BLED :___/___/___ BLED BY: _____

CONTACT NAME: _____ Telephone (___) ____-_____

NAME OF ALTERNATE: _____ Telephone (___) ____-_____

WING BAND NUMBER IN SEQUENCE	REMARKS OR STATUS ("New" dead, missing, etc.) For new birds to flock, list the number and state "new bird"	WEE	SLE	WNV

Remarks:(After bird has been reported dead, put band number and list as "old dead" or "old missing" in this space)

Date received by VRDL: ____/____/____. Tested: ____/____/____. Reported to agency contact:____/____/____

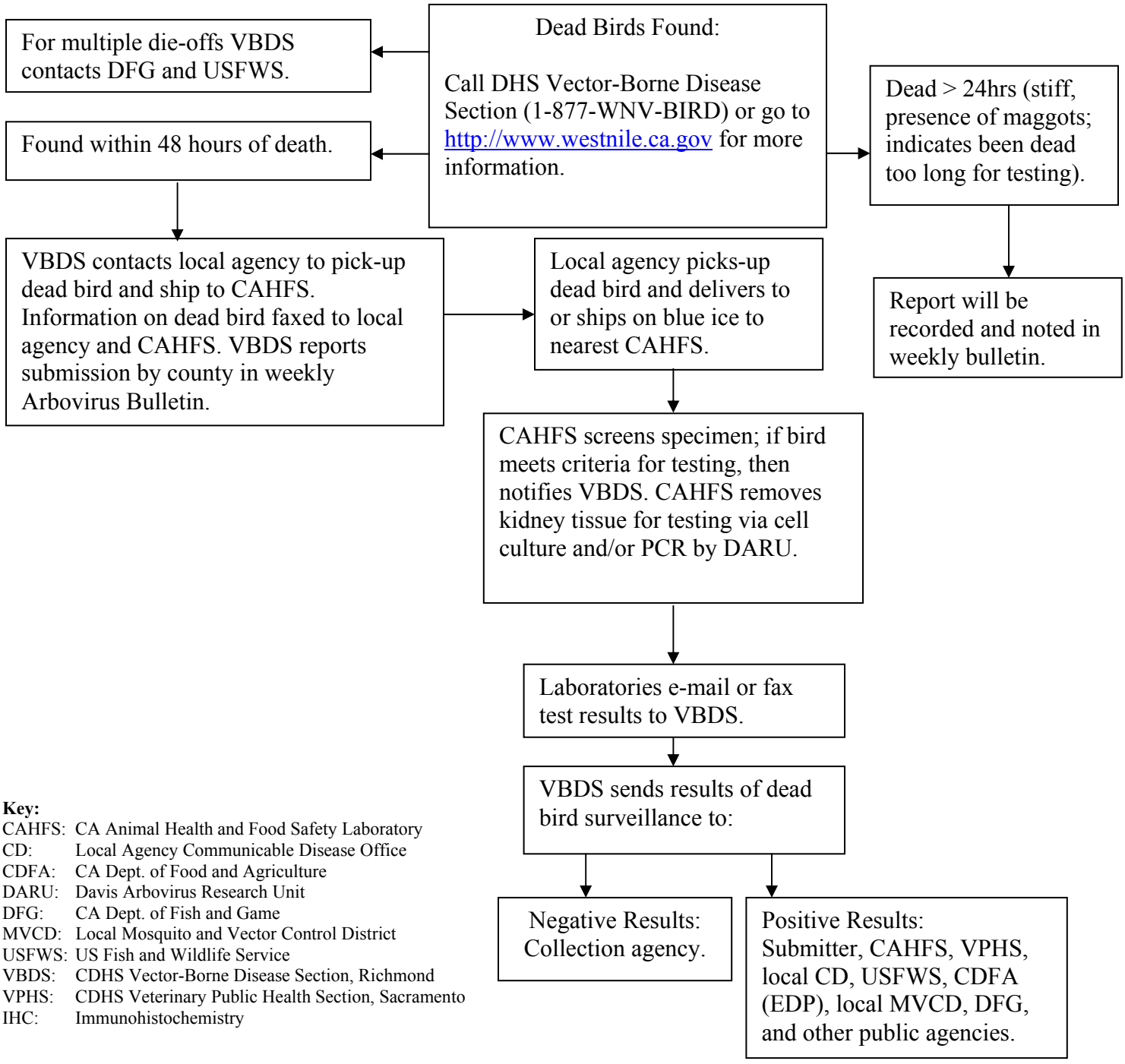
List all birds that have been in the flock.

A COPY OF THIS FORM MUST ACCOMPANY ALL SHIPMENTS OF CHICKEN BLOOD TO VRDL. ANY FUTURE SHIPMENTS FROM THIS SITE MUST USE THE SAME SITE CODE. TO REGISTER A NEW SITE, FAX A COPY OF THE SITE REGISTRATION FORM (MBVS-1) TO (530) 754-6360 OR E-MAIL TO bfeldridge@ucdavis.edu, UC DAVIS.

Form MBVS-2

Appendix D: Procedures for Testing Dead Birds

In mid-2000, DHS initiated a dead bird surveillance program in collaboration with other public agencies. DHS annually notifies about 600 agencies, organizations, and veterinarians involved with wildlife, including rehabilitation centers, about the program. Dead birds are reported to DHS, shipped to a California Animal Health & Food Safety Laboratory for screening and removal of kidney tissue, which is then sent to the UC Davis Arbovirus Research Unit for WN viral isolation. The dead bird testing algorithm is provided below.



Key:
CAHFS: CA Animal Health and Food Safety Laboratory
CD: Local Agency Communicable Disease Office
CDFA: CA Dept. of Food and Agriculture
DARU: Davis Arbovirus Research Unit
DFG: CA Dept. of Fish and Game
MVCD: Local Mosquito and Vector Control District
USFWS: US Fish and Wildlife Service
VBDS: CDHS Vector-Borne Disease Section, Richmond
VPHS: CDHS Veterinary Public Health Section, Sacramento
IHC: Immunohistochemistry

Dead Bird Submission Instructions for Local Agencies
California West Nile Virus (WNV) Dead Bird Surveillance Program
California Department of Health Services (DHS)
Division of Communicable Disease Control

Dead Bird Reporting and Submission Instructions for Local Agencies

When your agency receives a call from the public about a dead bird (especially recently dead crows, ravens, magpies, jays, or raptors), or one of your staff members finds a dead bird, please immediately refer them to the DHS Hotline at 1-877-WNV-BIRD (877-968-2473).

DHS will assess the suitability of the dead bird for testing and contact your agency only if the bird is approved for pickup. The WNV Hotline is monitored 8am-4pm Monday through Friday. Any dead birds sent without prior notification will not be tested.

Once the dead bird submission is approved, DHS will arrange for the pick-up of the carcass to be shipped from your agency to the nearest California Animal Health and Food Safety Laboratory (CAHFS). Dead birds sent to CAHFS San Bernardino, Turlock, and Fresno laboratories will then be transported by CAHFS to Davis. In 2003, all WNV testing will be conducted at CAHFS Central and the Davis Arbovirus Research Unit (DARU). CAHFS will perform screening tests, remove specific tissues, and forward tissues to DARU for viral assay. Shipping and testing expenses will be paid by DHS.

To ensure the proper condition of specimen for testing and to comply with regulations for shipping diagnostic specimens, please follow these instructions.

Bird Carcasses

- Only dead birds can be picked up according to our permit.
- Do not touch the carcass with bare hands: wear rubber or latex gloves when picking up and handling it. If gloves are not available, use a plastic bag turned inside out over your hand, and invert the bag to surround the bird.
- Only agencies listed under the permit issued to DHS from the California Department of Fish and Game and U.S. Fish and Wildlife Service are authorized to pick up dead birds. The agencies covered include local mosquito abatement districts, some environmental health departments, and other designated agencies.
- **Collect recently dead birds.** Badly decomposed or scavenged carcasses are of limited diagnostic value. Signs that a bird has been dead for too long (over 48-72 hours) are the presence of many maggots, an extremely light weight carcass,

missing eyes, skin discoloration, skin or feathers rub off easily, strong odor, or a soft, mushy carcass.

- **If upon pick-up the carcass is found to be unacceptable (wrong species or badly decomposed), please collect the bird and dispose of it by placing it inside a double bag (tie or zip lock) and place it in a secure garbage can or dumpster.** California Department of Fish and Game and the U.S. Fish and Wildlife Service prefer that your burn or bury the carcass, but disposing of it in a dumpster is acceptable. **Immediately call DHS and notify them that the bird will no longer be tested so that we can remove the bird from the "submitted" category.**

- Place each bird carcass into a plastic bag and secure it inside a second plastic bag and zip lock it shut. **Double bagging prevents cross contamination and leakage**. **There should always be two bags separating the bird from documents/ labels that accompany it during shipping.**

- **Pack the bird carcass with blue ice packs.** An absorbent material, such as newspaper, must be included in the box to prevent any leakage from the box in accordance with shipping regulations.

- **Enclose the shipping document into a SEPARATE ZIP-LOCK BAG. Information includes a return-address label, so your box can be returned, and a copy of the dead bird submission form (with the dead bird number) faxed by DHS.** CAHFS prefers you put this separate zip-lock bag inside the outer bag containing the dead bird.

- Ship the bird carcass in a hard-sided plastic cooler or a styrofoam cooler placed in a cardboard box. If there is space between the Styrofoam cooler and cardboard box, fill the space with wadded newspaper. Unprotected styrofoam containers may break into pieces during shipment. **Notify DHS to arrange for carrier pickup to ship Monday through Thursday. This guarantees arrival at CAHFS before the weekend. Birds collected on Friday (from counties within overnight shipping distance from Davis) may be shipped to CAHFS Central as they are open on Saturday.**

- Birds that need to be stored over the **weekend** should be put on dry ice or stored at -70ºC. Freezing the carcass lessens the quality of tissue samples. **Refrigerating** the carcass is recommended for **overnight storage** (this slows virus deterioration, but does not stop it). **DO NOT store the carcass in a regular freezer** (usually -20º) at any time.

- Label the outside of the package with the words **Diagnostic Specimens ATTN: WNV** above the designated CAHFS address.

Dead Bird Shipping List

In preparation for the arrival of West Nile Virus, please verify that your district has the following items:

- CAHFS Addresses (see below)
- Crumpled newspapers or another absorbent material
- Rubber or Latex Gloves
- Dead Bird Shipping Boxes
  - inner zip-lock bag
  - outer zip-lock bag
  - Inner Styrofoam box
  - Outer Cardboard box
- Brown Shipping Paper
- DHS Phone Number: 877-WNV-BIRD (manned 8am-4pm weekdays).

CAHFS Central Laboratory (530) 752-8709
ATTN: WNV
Dr. Leslie Woods
University of California, Davis
West Health Science Drive
Davis, CA 95616

CAHFS San Bernardino (909) 383-4287
ATTN: WNV
Dr. Deryck Read
105 West Central Avenue
San Bernardino, CA 92412

CAHFS Fresno (559) 498-7740
ATTN: WNV
Dr. Richard Chin
2789 South Orange Avenue
Fresno, CA 93725

CAHFS Turlock (209) 634-5837
ATTN: WNV
Dr. Bruce Charlton
P.O. Box 1522
Fulkerth & Soderquist Road
Turlock, CA 95381

For Veterinarians:
If your laboratory will be performing necropsies, please ship the following tissues in a pooled sample: brain, kidney, heart and spleen. DHS prefers tissues be sent in cryovials on dry ice in a hard plastic or Styrofoam container placed in a cardboard box. If cryovials are not available, use any screw cap tubes. If dry ice is not available, please use wet ice in shipping container. If using wet ice, please ensure that samples are contained in watertight tubes so that water from melting ice does not leak into tissues. To avoid crushing specimen during shipping, please wrap specimen containers in sufficient padding such as bubble wrap.

Appendix E: Procedures for Testing Equines and Ratites

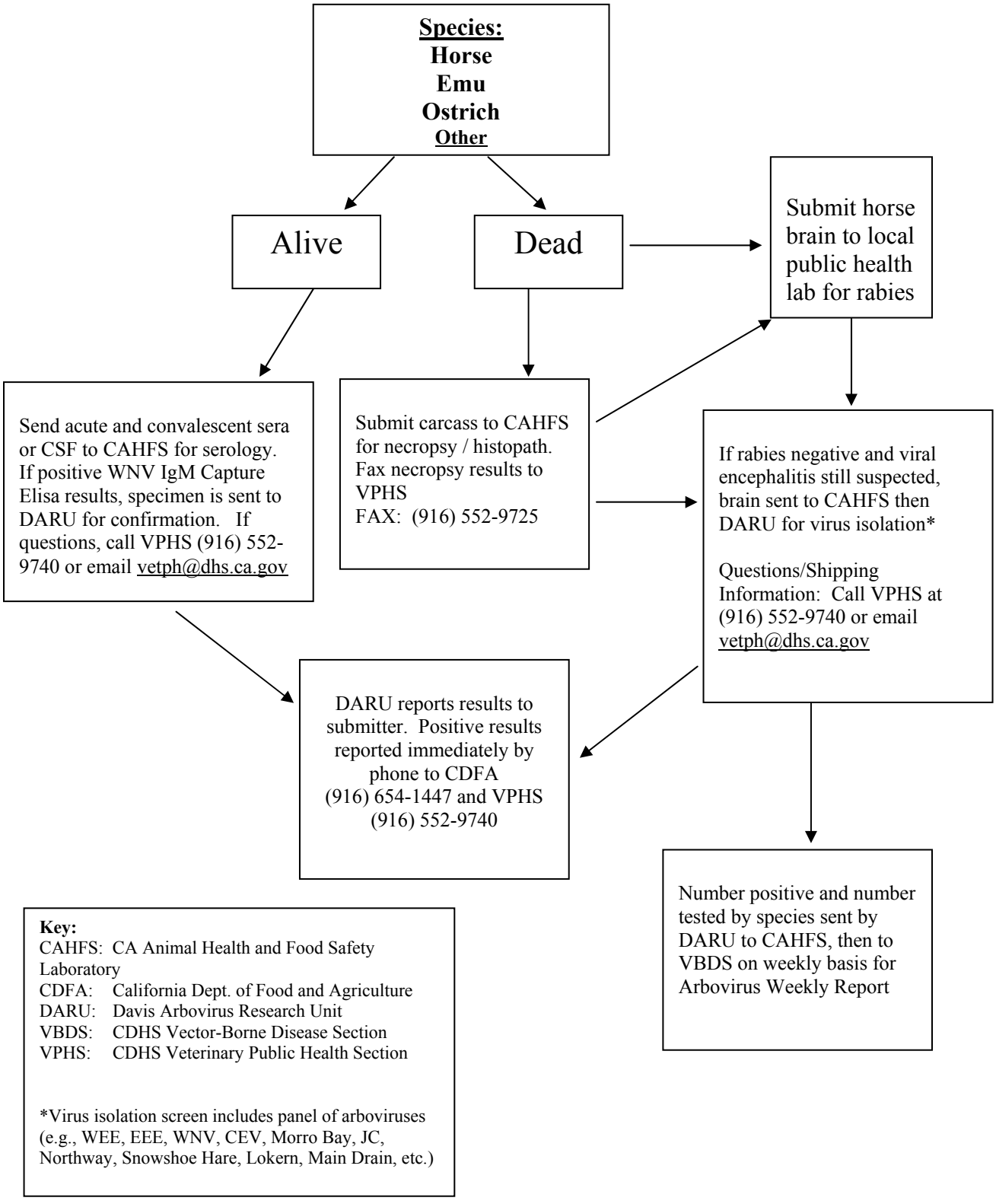
The California Department of Health Services (DHS) and the California Department of Food and Agriculture (CDFA) have a well-established passive surveillance program for equine and ratite encephalomyelitis. Equine encephalomyelitides is legally reportable to CDFA by veterinarians and diagnostic laboratories pursuant to Section 9101 of the Food and Agricultural Code. Venezuelan equine encephalitis and West Nile virus are emergency animal diseases that must be reported to CDFA by telephone within 24 hours.

This appendix contains a copy of the mailing sent to veterinarians, public health lab directors, local health officers, public health veterinarians, animal health branch personnel, and interested parties every spring to inform them about the California Equine and Ratite Arbovirus Surveillance Program. The mailing includes a case definition for equine encephalomyelitides and instructions for specimen collection and submission for both equine and ratite samples. The mailing is distributed to approximately 1,200 practitioners, equine organizations, and other interested parties. Specimen submission is coordinated through the California Animal Health and Food Safety Laboratory System's (CAHFS) five regional branches, the University of California at Davis (UCD) School of Veterinary Medicine's Veterinary Medical Teaching Hospital, and other laboratories or individual veterinarians. Initial equine testing is performed by CAHFS, using the ELISA test for WNV IgM. Any positive results are sent to the UCD Center for Vector-Borne Disease Research, Arbovirus Research Unit (DARU) for confirmation, using virus isolation and identification of a large panel of arboviruses (WEE, EEE, WN, California encephalitis, Morro Bay, Jamestown Canyon, Northway, Snowshoe Hare, Lokern, Main Drain) and serology using the plaque reduction neutralization test (WEE, EEE, WN, SLE). All fatal cases of equine encephalitis are first tested for rabies at the local public health laboratory. An algorithm outlining the protocol for specimen submission and reporting is available for participants in the program.

Outreach is an important component of the program. DHS and CDFA have developed and distributed educational materials concerning the diagnosis and reporting of arboviruses in equines and ratites. DHS and CDFA work closely with equine veterinary referral centers, the California Horse Racing Board, and other interested parties to improve surveillance and reporting of suspect cases of equine and ratite encephalomyelitides.

Additional information on WN virus for veterinarians, horse owners, and ratite owners, including a fact sheet on the equine West Nile virus vaccine, is available at CDFA's website: http://www.cdfa.ca.gov/ahfss/ah/wnv_info.htm. A brochure containing facts about California WNV surveillance and general information about prevention and control is available at DHS's website: http://www.westnile.ca.gov.

Algorithm for Submission of Specimens from Domestic Animals with Neurologic Symptoms



STATE OF CALIFORNIA

DEPARTMENT OF FOOD AND AGRICULTURE
ANIMAL HEALTH AND FOOD SAFETY SERVICES
ANIMAL HEALTH BRANCH
1220 N STREET, ROOM A-107
SACRAMENTO, CA 95814
(916) 654-1447

DEPARTMENT OF HEALTH SERVICES
DIVISION OF COMMUNICABLE DISEASE
VETERINARY PUBLIC HEALTH SECTION
1616 Capitol Ave, M/S 7308
P. O. BOX 942732
SACRAMENTO, CA 94234-7320
(916) 552-9740

April 2003

To: California Veterinarians in Large Animal Practice
Public Health Laboratory Directors
Local Health Officers
Public Health Veterinarians
Animal Health Branch Personnel
Interested Parties

Subject: SURVEILLANCE AND REPORTING OF ARBOVIRAL ENCEPHALITIS VIRUSES IN HORSES AND RATITES

The California Department of Health Services (DHS) and the California Department of Food and Agriculture (CDFA) provide **free diagnostic testing on clinically affected horses for arboviral encephalitis viruses.** These include western equine encephalitis (WEE), eastern equine encephalitis (EEE), and West Nile virus (WNV). These diseases may affect horses, ratites (ostriches, emus, rheas, etc.), humans, and other birds and mammals. Your continued support of the surveillance program in California is important to both human and animal health. **Equine specimen submission instructions are provided in Attachment A.** Ratite specimen submissions should be coordinated through a California Animal Health and Food Safety (CAHFS)* Laboratory in your area (see attachment).

Veterinarians are often the first to detect the emergence of zoonotic diseases such as WNV. There were 14,717 equine clinical cases of WNV reported the United States in 2002. Mortality (dead or euthanized horses) was approximately 30%. To date, horses have not become infected with WNV while in California. However, based on the rapid spread of the virus westward, it is predicted that WNV will arrive this year via infected mosquitoes, birds, or mammals. Additionally, there have been significant levels of WEE virus activity in sentinel chickens and mosquitoes in recent years. Therefore, continued vigilance on the part of veterinarians, public health officials, and animal keepers is critical.

The decision to vaccinate for WNV, WEE and EEE should be based on a mosquito-exposure risk assessment analysis between the veterinary practitioner and horse owner. It is important to emphasize that once a horse has developed encephalitis, vaccination provides no benefit. Combination equine encephalitis vaccines should not be used in suspect cases because vaccination titers may interfere with diagnostic tests. Accurate vaccination records should be maintained because it is important to distinguish between vaccinated and exposed or infected horses. In addition, international shipments of horses with WNV titers may be restricted.

The WNV vaccine is now fully licensed by United States Department of Agriculture, and the manufacturer recommends two doses, three weeks apart, plus annual revaccination. Effectiveness has been demonstrated three weeks after the administration of the second of the two doses. Therefore, the initial two dosages should be administered at least three weeks prior to mosquito season. There is also now evidence that 6-month booster vaccinations are likely to enhance protection. It appears that one dose does not provide protective immunity because several horses in endemic areas became infected with WNV despite receiving a single dose of the vaccine.

In addition to vaccination, it cannot be overemphasized that the best prevention for WNV, WEE, and EEE includes eliminating or drastically minimizing the mosquito exposure to the horse. This involves the elimination of mosquitoes and their breeding grounds composed of standing stagnant water. Approved mosquito repellants are also indicated if exposure is unavoidable.

Your participation in this important public health program is greatly appreciated. For more information on WNV and other equine encephalitis viruses, please visit our Web site at http://westnile.ca.gov. If you require additional information, please contact your CDFA, Animal Health Branch District Office (see attachment) or the Veterinary Public Health Section of DHS at (916) 552-9740.

Kenneth L. Thomazin, D.V.M	Michele Jay-Russell, D.V.M., M.P.V.M.
Chief	Acting Chief
Animal Health Branch	Veterinary Public Health Section

Attachments

cc: Alex Ardans, D.V.M., M.S., Director
California Animal Health and Food Safety Laboratory System

Thomas W. Scott, Ph.D., Director
Davis Arbovirus Research Unit
Center for Vector-Borne Disease Research
University of California at Davis

*** See CAHFS attachment for locations and addresses**

SURVEILLANCE CASE DEFINITION FOR CONFIRMED WEST NILE VIRUS INFECTION IN EQUINES

NOTE: A HORSE WITH SIGNS OF ENCEPHALITIS MAY HAVE RABIES – TAKE PROPER PRECAUTIONS

Confirmed Case:

A horse with compatible clinical signs including ataxia (stumbling, staggering, wobbly gait, or incoordination) or at least two of the following: circling, hind limb weakness, inability to stand, multiple limb paralysis, muscle fasciculation, proprioceptive deficits, blindness, lip droop/paralysis, teeth grinding, acute death.

Plus one or more of the following:

- Isolation of West Nile (WN) virus from tissues[1]
- An associated 4-fold or greater change in plaque-reduction neutralization test (PRNT) antibody titer to WN virus in appropriately timed[2], paired sera
- Detection of both IgM antibody to WN virus by IgM-capture ELISA in serum or cerebrospinal fluid (CSF) and an elevated titer (1:10 or greater to WN virus antibody by PRNT in serum;
- Detection of both IgM antibody to WN virus by IgM-capture ELISA in serum or CSF and a positive polymerase chain reaction (PCR)[3] for WN virus genomic sequences in tissues[1]
- Detection of both IgM antibody to WN virus by IgM-capture ELISA in serum or CSF and a positive immunohistochemistry (IHC) for WN virus antigen in tissue;
- Positive IHC for WN virus antigen in tissue and a positive PCR[3] for WN virus genomic sequences in tissues.

Probable Case[4]:

Compatible clinical signs plus one of the following:

- Detection of IgM antibody to WN virus by IgM-capture ELISA in serum or CSF, but no elevated titer (negative at 1:10) to WN virus antibody by PRNT in serum
- No positive PCR[3] for WN virus genomic sequences tissues, and no positive IHC for WN virus antigen in tissue;
- Positive PCR[3] for WN virus genomic sequences in tissues;
- Positive IHC for WN virus antigen in tissue.

[1] Preferred diagnostic tissues from equine are brain or spinal cord; although tissues may include blood or CSF, the only known reports of WN virus isolation or positive PCR from equine blood or CSF have been related to experimentally infected animals.
[2] The first serum should be drawn as soon as possible after onset of clinical signs and the second drawn at least seven days after the first.
[3] For horses it is recommended that rt-nested polymerase chain reaction assay be used to maximize sensitivity of the test (Emerg Infect Dis. 2001 Jul-Aug; 7(4):739-41)
[4] An equine case classified as a suspect or probable case should, if possible, undergo further diagnostic testing to confirm or rule out WN virus as the cause of the clinical illness

Suspect Case[4]:

Compatible clinical signs

Assumptions on which case definition is based:

- Antibody in serum may be due to vaccination or a natural exposure; additional testing must be done to confirm WN virus infection in a vaccinated horse.
- IgM-capture ELISA testing may be slightly non-specific; cross-reactions to closely related flaviviruses (e.g., SLE virus) may occur.
- IgM antibody in equine serum is relatively short-lived; a positive IgM-capture ELISA means exposure to WN virus or a closely related flavivirus has occurred, very likely within the last three months.
- Neutralizing antibody, as detected by PRNT, may not be present in equine serum until two weeks or more after exposure to WN virus; it is possible that clinical signs may be present in an equine before a serum PRNT is positive.
- Neutralizing antibody detected in serum by PRNT indicates past exposure to WN virus; equines exposed to WN virus prior to 2002 may test positive for neutralizing antibody by PRNT.

PLEASE CONTACT CDFA OR DHS TO DISCUSS WEST NILE VIRUS TEST RESULTS FROM PRIVATE DIAGNOSTIC LABORATORIES

Attachment A

Protocol for Submission of Laboratory Specimens for Equine Neurological Disease Diagnosis and Surveillance
April 2003

1. Specimen collection and submission:

A. Blood

- Acute sample (5-10 ml) / no later than 7 days after onset
- Convalescent sample (5-10 ml) / 14-21 days after onset

Red top tubes of whole blood or serum (no preservatives or anticoagulants) should be submitted at ambient temperature to the California Animal Health and Food Safety (CAHFS) Laboratory* in your area. Do not freeze whole blood.

B. Brain

- Submission of the intact head is preferable because: 1) brain is better preserved (anatomically and virus titer) when left in the skull during transport, 2) specimens will be ruined if removal is not done correctly, and 3) brain removal in field conditions may increase the risk of exposure to rabies.
- **The intact head should be chilled immediately after removal. Submit it to a CAHFS Laboratory* in your area as quickly as possible.** Prepare a leak proof insulated transporting container with "cold packs" to keep the specimen at 4^{o} C while in transit. *When it is impossible for the CAHFS Laboratory to receive the chilled intact head within 48 hours, the submission protocol should be coordinated with the lab.*
- Specimens will then be forwarded by CAHFS to 1) a Public Health Laboratory to confirm or rule out rabies, and 2) The California Vector Borne Disease Laboratory (CVBRD) for arboviral testing. *In addition, brain will be examined microscopically for changes compatible with viral encephalitis or other causes of neurologic disease.*

C. Other specimens for differential neurological diagnoses

- Protocol for submission of serum, CSF or carcasses may be coordinated through CAHFS*.

2. Submission forms: Complete and include the transmittal forms supplied by the CAHFS. See attached sample or download the form from their Website: http://cahfs/ucdavis.edu The submittal form for each specimen should be placed in a leak proof plastic bag and attached to the corresponding container.

3. Shipment: Check with the CAHFS Laboratory in your area for assistance with shipping regulations governing the transportation of infectious materials.

* See CAHFS attachment for locations and addresses

Appendix F: Protocol for Submission of Laboratory Specimens for Human Disease and Surveillance

Specimens will be accepted on cases that meet one of the following case definitions:

A. ***Viral Encephalitis**** characterized by:
- Encephalopathy (depressed or altered level of consciousness, lethargy, or personality change), and one or more of the following:
- Fever (T≥38C), seizure(s), focal neurologic findings, CSF pleocytosis, abnormal EEG, abnormal neuroimaging

B. ***Aseptic meningitis*** (patients ≥ 17 years of age) characterized by:
- Fever (T≥38C), headache, stiff neck and/or other meningeal signs
- CSF pleocytosis

C. ***Acute Flaccid Paralysis/Atypical Guillain-Barré Syndrome*** characterized by:
- Fever (T≥38C), altered mental status, and/or CSF pleocytosis

Specimens

Required

- **Acute Serum:** ≥ 2cc serum collected ≤ 7 days after
- **Cerebral Spinal Fluid:** 1-2cc CSF *if lumbar puncture was performed*
- **Convalescent Serum:** ≥ 2 cc serum collected 10-14 days after onset

Contact your local health department for instructions on where to send specimens.

Appendix G: Surveillance Case Definition for West Nile Virus Infection in Humans

(Modified from: "CDC. Epidemic/Epizootic West Nile Virus in the United States: Guidelines for Surveillance, Prevention, and Control" at www.cdc.gov/ncidod/dvbid/westnile/publications.htm)

Confirmed case: A confirmed case of West Nile illness is defined as a febrile illness associated with neurologic manifestations ranging from headache to aseptic meningitis or encephalitis, plus at least one of the following:

- Demonstration of both WN virus-specific IgM (by EIA) and IgG (screened by EIA and confirmed by PRNT) antibody in a single serum specimen;
- A 4-fold serial change in plaque-reduction neutralizing (PRNT) antibody titer to WN virus in paired, appropriately timed serum or CSF samples;
- Demonstration of IgM antibody to WN virus in CSF by IgM capture EIA;
- Isolation of WN virus from, or demonstration of WN viral antigen or genomic sequences in tissue, blood, CSF, or other body fluid.

Probable case: A probable case is defined as a compatible illness (as above) that does not meet any of the above laboratory criteria, plus at least one of the following:

- Demonstration of serum IgM antibody against WN virus (by EIA);
- Demonstration of an elevated titer of WN virus-specific IgG antibody in convalescent-phase serum (screened by EIA and confirmed by PRNT).

Non-case: A non-case is defined as an illness that does not meet any of the above laboratory criteria, plus:

- A negative test for IgM to WN virus (by EIA) in serum or CSF collected 8-21 days after onset of illness;

And/or

- A negative test for IgG antibody to WN virus (by EIA or PRNT) in serum collected >22 days after onset of illness.

PLEASE CONTACT DHS AT (510) 307-8606 TO DISCUSS WN TEST RESULTS FROM PRIVATE DIAGNOSTIC LABORATORIES.

Appendix H: Compounds Approved for Mosquito Control in California

Label rates and usage vary from year to year and geographically; consult your County Agricultural Commissioner and the California Department of Fish and Game before application. Examples of products containing specific active ingredients are provided below, but this is not an inclusive list nor constitutes product endorsement. For more information on pesticides and mosquito control, please refer to the Environmental Protection Agency (EPA) Web site:
http://www.epa.gov/pesticides/factsheets/skeeters.htm

Larvicides:

1. *Bacillus thuringiensis* subspecies *israelensis* (Bti: e.g. Aquabac 200G, VectoBac® 12AS, Teknar HP-D)
   Use: Approved for most permanent and temporary bodies of water.
   Limitations: Only works on actively feeding stages. Does not persist well in the water column..

2. *Bacillus sphaericus* (Bs: e.g. VectoLex® CG)
   Use: Approved for most permanent and temporary bodies of water.
   Limitations: Only works on actively feeding stages. Does not work well on all species. May persist and have residual activity in some sites.

3. IGRs (Insect Growth Regulators)
   a. (S)-Methoprene (e.g. Altosid® Pellets)
   Use: Approved for most permanent and temporary bodies of water.
   Limitations: Works best on older instars. Some populations of mosquitoes may show some resistance.
   b. Diflurobenzamide (e.g. Dimilin®25W)
   Use: Impounded tailwater, sewage effluent, urban drains and catch basins.
   Limitations: Cannot be applied to wetlands, crops, or near estuaries.

4. Larviciding oils (e.g. Mosquito Larvicide GB-1111)
   Use: Ditches, dairy lagoons, floodwater. Effective against all stages, including pupae.
   Limitations: Consult with the California Department of Fish and Game for local restrictions.

5. Monomolecular films (e.g. Agnique® MMF)
   Use: Most standing water including certain crops.
   Limitations: Does not work well in areas with unidirectional winds in excess of ten mph.

Adulticides:

1. Organophosphate compounds
   Note: Many *Cx. tarsalis* populations in the Central Valley are resistant to label OP application rates.
   a. Malathion (e.g. Fyfanon® ULV)
   Use: May be applied by air or ground equipment over urban areas, some crops including rice, wetlands.
   Limitations: Paint damage to cars; toxic to fish, wildlife and bees; crop residue limitations restrict application before harvest.
   b. Naled (e.g. Dibrom® Concentrate, Trumpet® EC)
   Use: Air or ground application on fodder crops, swamps, floodwater, residential areas.
   Limitations: Similar to malathion.

2. Pyrethrins (natural pyrethrin products: e.g. Pyrenone® Crop Spray, Pyrenone® 25-5)
   Use: Wetlands, floodwater, residential areas, some crops.
   Limitations: Do not apply to drinking water, milking areas; may be toxic to bees, fish, and some wildlife. Some formulations with synergists have greater limitations.

3. Pyrethroids (synthetic pyrethrin products containing deltamethrin, permethrin, resmethrin or sumithrin: e.g. Suspend® SC, Aqua-Reslin®, Scourge® Insecticide, Anvil® 10+10 ULV)
   Use: All non-crop areas including wetlands and floodwater.
   Limitations: May be toxic to bees, fish, and some wildlife; avoid treating food crops, drinking water or milk production.

Appendix I. Websites Related to Weather Conditions and Forecasts, Crop Acreage and Production, Mosquito Control, and Arbovirus Surveillance In California

Website	URL	Available information
California Data Exchange Center	http://cdec.water.ca.gov	Water-related data from the California Department of Water Resources, including historical and current streamflow, snowpack, and precipitation information.
UC IPM Online	http://www.ipm.ucdavis.edu	Precipitation and temperature data for stations throughout California; also allows calculation of degree-days based on user-defined data and parameters.
National Weather Service – Climate Prediction Center	http://www.cpc.ncep.noaa.gov/products/predictions/	Short-range (daily) to long-range (seasonal) temperature and precipitation forecasts. Also provides El Niño-related forecasts.
California Agricultural Statistics Service	http://www.nass.usda.gov/ca/	Crop acreage, yield, and production estimates for past years and the current year's projections. Reports for particular crops are published at specific times during the year – see the calendar on the website.
West Nile Virus – California Department of Health Services	http://westnile.ca.gov	Online dead bird reporting and general information on West Nile virus, mosquito control, and other related issues in California.
California Vectorborne Disease Surveillance System	http://vector.ucdavis.edu	Frequently updated reports and maps on arbovirus surveillance and mosquito occurrence in California.
Mosquito and Vector Control Association of California	http://www.mvcac.org	News, membership information, event calendars, and other topics of interest to California's mosquito control agencies.